

~~32. A composition comprising SNO-Hb[FeII].~~

33. A method for preparing a composition comprising SNO-Hb[FeII]O₂, said method comprising incubating excess S-nitrosocysteine, S-nitrosohomocysteine, S-nitrosocysteinylglycine, or S-nitrosoglutathione with purified hemoglobin in the presence of oxygen at a pH of about 7.4 to about 9.2.
34. A method for preparing a composition comprising SNO-Hb[FeII], said method comprising incubating excess S-nitrosocysteine, S-nitrosohomocysteine, S-nitrosocysteinylglycine, or S-nitrosoglutathione with purified hemoglobin in the absence of oxygen at a pH of about 7.4 to about 9.2.

REMARKS

Claims 11 and 14 have been canceled. Claims 5, 10, 13, 15, 16 and 18-23 have been amended. Claims 30-34 have been added.

Support for Claim 30 can be found in the specification on page 10, line 30 to page 11, line 19, on page 12, lines 9-15, on page 13, line 7 to page 14, line 3, and in the original Claim 15, for instance.

Support for Claim 31 can be found, for example, on page 11, line 29 to page 12, line 2.

Support for Claim 32 can be found, for example, on page 12, lines 3-8.

Support for Claim 33 is found on page 19, lines 8-21 and on page 20, lines 30-34, for example.

Support for Claim 34 is found on page 18, line 27 to page 19, line 6, for instance.

Support for amendments to Claim 5 can be found in the specification on page 22, lines 17-24, for example.

Outstanding Objections and/or Rejections

The Examiner states, on page 2 of the Office Action of August 26, 1999, "The outstanding objection(s) and/or rejection(s) as presented in the final office action in paper no. 18 (dated 6/5/98) are still in force in their entirety." These outstanding objections and rejections are

not discussed below, as the Examiner states that he will consider Applicant's arguments directed to these rejections as presented in the Brief on Appeal.

Comments Regarding Examiner's Discussion of Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132; Comments Apply to All Rejections in Which WO 93/09806 Has Been Cited

The Examiner concludes that the reagent used in experiments to attempt the synthesis of SNO-hemoglobin was SNOAc. The Examiner bases this conclusion on page 58, lines 17-25 of Example 19 in WO 93/09806 and on Figures 1 and 2 of 08/559,172, now abandoned, which the Examiner observes are the same spectra as Figures 28 and 29 of WO 93/09806. It should be noted that in the interview conducted on August 5, 1999 at the United States Patent and Trademark Office, Dr. Stamler stated that the Examples in WO 93/09806 and 08/559,172 were attempts to describe the same experiments, and that both Examples were inaccurate in similar ways. Among the inaccuracies was the identification of the reagent in the first paragraph of the Examples as SNOAc, when, according to Dr. Stamler, the reagent used in that experiment was acidified nitrite. See Exhibits A and B accompanying the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 mailed to the United States Patent and Trademark Office on February 17, 1998. Thus, it is not appropriate to cite 08/559,172 as proof that SNOAc is the correct reagent. The Examiner should also note that the method to allegedly produce S-nitrosohemoglobin appearing in Example 19 of WO 93/09806 and Example 1 of 08/559,172 was not published in a scientific journal, but the method described in the subject patent application was published in the refereed journal *Nature* in March, 1996, shortly after the filing date of the subject patent application [Jia, L. *et al.*, *Nature* 380:221-226 (21 March 1996)].

In the second paragraph of the discussion of the Declaration, the Examiner sets a new standard for Applicant to meet in the Examiner's determination of whether a reference sets forth an enabling description of an invention, with the requirement that the Applicant prove the absence of SNO-hemoglobin. This standard is impossible to meet; it is impossible to prove the complete absence of anything.

The standard for determining whether a prior art reference is enabling has been set forth in *In re Donahue* (226 USPQ 619, 621).

It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it. *In re Sasse*, 629 F.2d 675, 681, 207 USPQ 107, 111 (CCPA 1980); *In re Samour*, 571 F.2d at

562, 197 USPQ at 4; see also *Reading & Bates Construction Co. v. Baker Energy Resources Corp.*, 748 F.2d 64, 651-52, 223 USPQ 1168, 1173 (Fed. Cir. 1984). Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his own knowledge to make the claimed invention. See *In re Grice*, 301 F.2d at 939, 133 USPQ at 373-74. Accordingly, even if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. *In re Borst*, 345 F.2d 851, 855, 45 USPQ 554, 557 (CCPA 1965), *cert. denied* 382 U.S. 973, 148 USPQ 771 (1966).

"When the reference relied upon expressly anticipates or makes obvious all of the elements of the claimed invention, the reference is presumed to be operable." MPEP 2121. *In re Sasse*, 629 F.2d 675, 207 USPQ 107 (CCPA 1980). Applicant's task after a 102 or 103 rejection has been to rebut, by a declaration, the presumption of operability by a preponderance of the evidence. Applicant has met this burden.

It should be remembered here that the beginning words of 35 U.S.C. § 102 read: "A person shall be entitled to a patent unless --" Thus, the primary burden of proof rests on the Patent Office to show that Applicant is not entitled to a patent. The Patent Office cannot impose a higher standard of proof on Applicant to rebut statements of the Patent Office than the Patent Office uses in producing those statements. See, for example, the statement in *In re Hoeksema* 158 USPQ 596, 601 (CCPA 1968); see footnote:

We think this approach to be eminently fair to all parties and in accord with the opinion of the Supreme Court in *Graham*, in its requiring that all of the pertinent evidence be considered while yet leaving the primary responsibility for sifting out unpatentable material with the Patent Office. *Graham v. John Deere Co.*, 383 U.S. 1 at 18, 148 USPQ at 467.

It would be practically impossible for an applicant to show that all known processes are incapable of producing the claimed compound.

The Examiner goes on to say in his discussion of the Declaration:

Applicant's own specification demonstrates that reacting a low molecular weight S-nitrosothiol such as SNOAc in equimolar amounts with hemoglobin (e.g. deoxy or oxy) would be expected to generate SNO-hemoglobin (e.g. see present specification at pages 46-48 and Figures 1a-1d). It is also noted that use of extrinsic evidence by the Examiner to demonstrate inherency is permitted (e.g. see

MPEP 2131.01(d)), including the use of applicant's own specification (e.g. examples). See *Ex parte Novitski*, 26 USPQ2d 1389 (B.P.A.I., 1993).

It is assumed the Examiner meant to say pages 17-19 instead of pages 46-48, in describing results shown in Figures 1a - 1d. It is not clear to Applicant how inherency is being used. The Examiner has not recited specific claims, or a specific property or element of the invention that is allegedly inherent, nor has he provided reasoning to support a conclusion of inherency in that context. Rather, the Examiner is attempting to use the theory of inherency in his assessment of the persuasiveness of the Declaration of Jonathan S. Stamler, M.D. Under 37 C.F.R. § 1.132 on the question of whether WO 93/09806 presents an enabling description of a synthesis of SNO-hemoglobin. The theory of inherency does not apply in this assessment, only in making a rejection under 35 U.S.C. § 102 or § 103. In any case, a conclusion -- for example, that SNOAc and hemoglobin would be expected to generate SNO-hemoglobin -- cannot be inherent. A result of an untested method cannot be inherent; only what is *necessarily* true can be called inherent.

It is appropriate to consider a reference for whether it contains an enabling description only by considering the reference and other knowledge available to one of ordinary skill in the art at the time of Applicant's priority date. Applicant's specification was not available to one of ordinary skill in the art at the priority date.

A reference contains an "enabling disclosure" if the public was in possession of the claimed invention before the date of invention. "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his [or her] own knowledge to make the claimed invention." *In re Donohue*, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985).

The Examiner states, "The Declarant's attempt to reproduce the Reference Example 19 method was not found persuasive since it is unclear as to whether applicant is showing the absence of SNO-hemoglobin or the inability of the utilized assay to detect the presence of SNO-hemoglobin." Exhibits E1-E3 accompanying the Declaration of Jonathan S. Stamler, M.D. include a standard "curve" which demonstrates that the assay can be used to detect low amounts of S-nitrosothiol. Dr. Stamler has indicated in the Declaration that this improved assay is being used now in his laboratory to assay for SNO-hemoglobin and that the unimproved assay that was used several years earlier in an attempt to detect SNO-hemoglobin in Example 19 of WO

93/09806 could not have detected SNO-hemoglobin had it been made. Moreover, as Dr. Stamler further stated in the Declaration, an essential step to separate the product nitrosothiol from the reagent nitrosothiol was never performed. Thus, any assay performed in this manner in an attempt to detect nitrosothiol would give a false positive result. From the "analysis" presented in Example 19 of WO 93/09806, one of ordinary skill in the art could not conclude that SNO-hemoglobin had been made. Further, there is no guidance found in the prior art on how to produce an S-nitrosylated protein that retains its enzymatic activity or other physiological function.

The Examiner refers also to Example 1 and Figures 1 and 2 in 08/559,172 (now abandoned), as a document "which confirms the presence of a 'composition which comprises SNO-hemoglobin' within the scope of the presently claimed invention." Again the Examiner refers to 08/559,172 as showing "the formation of S-nitrosyl hemoglobin at this pH [6.9] in addition to higher pH optimums." The Declarant explained in the interview that 08/559,172 and WO 93/09806 described the same experiments and contained similar errors in the description and conclusions of those experiments. Thus, the Declaration should serve to rebut statements in 08/559,172 as well as in WO 93/09806.

The Examiner states that one would be motivated to optimize reaction parameters such as pH as a matter of course. One of ordinary skill in the art, concluding from Example 19 that synthesis of SNO-hemoglobin was not shown, would have no motivation to take up any experiments to produce SNO-hemoglobin or any guidance about what to try. It was not obvious to increase the concentration of the reagent, as it was thought that the increase in the NO^\bullet generated would cause too much oxidation of the hemoglobin. Dr. Stamler stated in the interview that it had been thought, before the time of the invention, that an acidic pH was desirable. It was thought that SNOAc would release NO^\bullet at alkaline pH, which would cause the oxidation of hemoglobin. Therefore, it was not obvious that a higher pH should be used to S-nitrosylate hemoglobin. It was first appreciated by Applicant that the rate of S-nitrosylation of hemoglobin is dependent on the conformational state of hemoglobin (T versus R) associated with an alkaline pH. See page 20, lines 19-34, for instance.

A Declaration of Joseph Bonaventura, Ph.D. Under 37 C.F.R. § 1.132 was mailed to the Patent and Trademark Office on March 12, 1998. The Examiner has not set forth in the Office Action of August 26, 1999, or in any previous Office Action or Advisory Action, any reasons

why this Declaration was found to be nonpersuasive on the issue of non-enablement of a method for producing SNO-hemoglobin described in WO 93/09806.

Rejection of Claims 9-15, 18-21, 26 and 27 Under 35 U.S.C. § 102(b), Or, In the Alternative, Under 35 U.S.C. §103(a)

Claims 9-15, 18-21, 26 and 27 have been rejected under 35 U.S.C. § 102(b) as anticipated by, or, in the alternative, under 35 U.S.C. §103(a) as obvious over Stamler *et al.*, WO 93/09806.

The Examiner states that WO 93/09806 discloses SNO-hemoglobin and therapeutic methods for its use. WO 93/09806 does not present an enabling description of a method for producing SNO-hemoglobin. SNO-hemoglobin also had not been reported previously in any publication. Thus, WO 93/09806 also cannot present an enabling description of any method for using SNO-hemoglobin. Example 19 of WO 93/09806 presents a number of errors in reporting the procedures that were followed, in the logic applied to the results, and in the conclusions that were drawn from those results. These errors are described in the paragraphs that follow.

One matter is the missing reagent on line 5 of page 58 of WO 93/09806. Although the context of Example 19 might suggest that the reagent is SNOAc (*S*-nitroso-*N*-acetylcysteine), the laboratory notebook records of Dr. Stamler show that the reagent used for the experiment, the results of which are shown in Figure 28, was actually acidified nitrite. See Exhibits A and B with the Declaration of Jonathan S. Stamler Under 37 C.F.R. § 1.132 mailed to the United States Patent and Trademark Office on February 17, 1998.

If we assume that the missing reagent used with hemoglobin was SNOAc, then the Saville assay as described in Example 19 of WO 93/09806 could not have yielded interpretable results. Lacking the further improvements later developed, the Saville assay as described by B. Saville [“A Scheme for the Colorimetric Determination of Microgram Amounts of Thiols,” *Analyst* 83:670-672 (1958)], is inadequate to detect SNO-hemoglobin. Example 19 includes no report of a separation step to separate the reagent, a low molecular weight S-nitrosothiol, from the product, which the Examiner presumes to be SNO-hemoglobin, a high molecular weight protein. Thus, the assay would give the misleading result, with SNOAc, and in all such cases where a low molecular weight S-nitrosothiol is used as a reagent, that an S-nitrosothiol product was formed, as the assay would detect the reagent. In fact, no separation step was performed, as

Dr. Stamler has stated in the Declaration. See the first paragraph of statement 5 of the Declaration.

A minor matter is the number given as the absorption maximum of 450 nm as reported on line 14, for Figure 28. It can be seen from Figure 28 that this maximum is 540; one might assume the units are nanometers for the x-axis; no units are given for the y-axis. However, a more important matter is that it is not reported what solution produced this spectrum. It is not recognizable as a spectrum of hemoglobin. SNO-hemoglobin has no characteristic spectrum that is distinguishable from any other species of hemoglobin, as one of skill in the art knows. If the spectrum in Figure 28 is intended to be that of the azo dye which is generated from nitrosothiol in the Saville assay, it is not informative, as the Saville assay requires measurements made both in the presence and absence of mercuric ions, to assay for S-nitrosothiol.

The sentence at lines 15-16 of page 58 makes no sense, as the "S-nitrosothiol bond formation" being referred to in this sentence is supposed to be in SNO-hemoglobin, and could not be "demonstrated" in any way by "using NO⁺ equivalents in the form of SNOAC."

The conclusions stated in the second paragraph on page 58 of WO 93/09806 are wrong. It is impossible to tell from Figure 29 which line of the spectrum in the region of approximately 540-580 nm can be attributed to a particular synthesis procedure, as the lines of the five different spectra in the figure are not identified, and the lines of the spectra overlap. In any case, that region of the hemoglobin spectrum is difficult to interpret in general, as several species of hemoglobin absorb in that range of wavelengths. Note, for example, that the spectrum of NO(FeII)hemoglobin in the region of 540-580 nm resembles that of oxyhemoglobin. See Exhibit Z. Thus, the relative contributions of each of these species to the various spectra cannot be determined from the figure. What is clear, however, is that Figure 29 shows multiple hemoglobin derivatives in which the redox metal sites are different.

Figure 30, referred to on page 58, lines 19-25 of WO 93/09806, is said to be the spectrum of nitrosyl-hemoglobin. However, Figure 30 is not helpful for purposes of comparison with any of the other spectra, being plotted as a separate graph.

Lines 23-25 of page 58 draw the conclusion, "The fact that the S-nitrosothiol did not react with the redox metal site of hemoglobin, but with its thiol group instead, indicates that the reactive NO species donated by the S-nitrosothiol is nitrosonium or nitroxyl." No such conclusion can be drawn. On the contrary, one skilled in the art would conclude that there was a

reaction with the redox metal site of hemoglobin. There is no evidence, from any of the assays or spectra examined, that S-nitrosothiol groups are present on a hemoglobin product, and no conclusion can be made about any reactive NO species from any of the experiments that might be described in Example 19.

Lines 26-27 of page 58 state, without any evidence, "S-nitrosylation of hemoglobin does not result in the formation of methemoglobin and consequent impairment in hemoglobin-oxygen binding." On the contrary, methemoglobin is definitely formed by the processes one might assume to be described in Example 19, as explained below.

The sentence on page 58, line 28 to page 59, line 1 describes "a leftward shift in the hemoglobin-oxygen association curve." This leftward shift was merely a result of the presence of methemoglobin. Experiments to produce a hemoglobin-oxygen association curve are done in a tonometer of various oxygen partial pressures. The presence of substantial amounts of methemoglobin ($\lambda_{\text{max}} = 405 \text{ nm}$) are well known to cause this shift. This has been misinterpreted in WO 93/09806 as an "increase in oxygen binding." The first sentence on page 59 continues, "Thus, the reaction between S-nitrosothiols and hemoglobin not only eliminates the inhibition of oxygen binding which occurs from the reaction with uncharged NO and generation of methemoglobin, but it actually increases oxygen binding." In the absence of any evidence of a reaction between S-nitrosothiols and the thiols of hemoglobin, one of ordinary skill in the art could not conclude what effect SNO-hemoglobin might have on oxygen binding.

The interpretation of the hemoglobin spectra in Figure 29 is not straightforward because of the many hemoglobin species with similar spectra. See, for example, the spectra of NO-hemoglobin in the Soret region and in the visible range (Exhibits Y and Z, respectively, which were produced in the laboratory of Dr. Stamler). There are, therefore, several alternative explanations that are more plausible to one of skill in the art, but all of them include reactions taking place at the metal redox center. None of the spectra of Figure 29 can be identified as being attributable to any one species of hemoglobin. To get an accurate measurement of the relative contributions of oxy-, NO(FeII)- or met-hemoglobin species among a mixture of hemoglobin species, it would be necessary to determine the amounts of the species oxy-, NO(FeII)-, and met-hemoglobin, using methods such as those reported by Gow, A.J. *et al.*, *Proc. Natl. Acad. Sci. USA* 96:9027-9032, 1999 (Exhibit X). This was not done in this case, and these methods were not known at the time of the publication of WO 93/09806. However, it is clear

that the products from all of the procedures 1-4 (as these procedures are summarized on Exhibit C with the Declaration of Dr. Stamler) include significant amounts of one or more species of hemoglobin that cause a leftward shift in the peaks of the Soret region of the spectrum. It is difficult to determine the exact maxima of the peaks, because the determinations were not made, or not given in Example 19. However, it is clear that the leftward shift from the "middle spectra" to those in spectra 3 and 4 is at least 5 nm, and that, overall, the peaks in the Soret region cover a range of at least 10 nm. The explanation for the leftwards shift is the presence of significant methemoglobin, as the maximum absorbance for oxyhemoglobin is about 415 nm, the maximum for NO-hemoglobin is about 417 nm, and the maximum for methemoglobin is about 405 nm. (The maximum for deoxyhemoglobin is 430 nm. At least one of the spectra would appear to have a partial deoxy component that would greatly confound the interpretation.) For procedures 1 and 2, the product has a maximum in the Soret region at 417 nm, which may indicate the presence of NO(FeII)hemoglobin [nitrosylhemoglobin], and for procedures 3 and 4, in which a higher concentration of SNOAc was used, there has been a leftwards shift, indicating that a major product is methemoglobin. The spectra cannot tell one of skill in the art anything about whether a reaction occurred at the thiols of the cysteine residues of hemoglobin; only that reactions are occurring at the redox metal center.

An alternative explanation for the leftward shift in the hemoglobin absorbance spectrum is that the methemoglobin present promotes the R (high affinity) structure. That is, if the hemes of one or more subunits of the hemoglobin tetramer are oxidized, the remaining subunits bind oxygen more readily than they would if the subunits were in the deoxy (T structure) state.

In any case, the experiment of procedures 1 and 2 of Exhibit C has been repeated, and the result was that, by an improved Saville assay modified from that known at the time of the publication of WO 93/09806, no SNO-hemoglobin was produced. See statement 5 of the Declaration of Dr. Stamler, and accompanying Exhibits E1-E3. WO 93/09806 does not contain a description of how each of the spectra in Figure 29 were derived. Figure 29 is, therefore, uninterpretable, but the conclusion of one of ordinary skill in the art could not be, seeing the maximum absorbance leftward shifted as it is, that (page 58, lines 17-19) "the UV spectrum of hemoglobin incubated with SNOAC shows no reaction at the redox metal (iron-binding site) of hemoglobin, over 15 minutes."

One of ordinary skill in the art looking for evidence that SNO-hemoglobin was made by the process in Example 19 would be left with nothing. The Example does not describe a credible assay used by persons of ordinary skill in the art -- quantitative or qualitative -- detecting the presence of SNO-hemoglobin. No spectrum characteristic of SNO-hemoglobin is presented -- or *can* be presented. Not one characteristic indicative of the presence of SNO-hemoglobin is given. No experiment showing a physiological effect of SNO-hemoglobin is shown. The “description” in WO 93/09806 includes discussion of SNO-hemoglobin as if it has been made, but, in reality, the “description” does not go beyond the mere naming of the compound, which does not constitute a description of the compound. “The mere naming of a compound in a reference, without more, cannot constitute a description of the compound, particularly when, as in this case, the evidence of record suggests that a method suitable for its preparation was not developed until a date later than that of the reference.” *In re Wiggins, James and Gittos*, 179 USPQ 421, 425.

As can be seen from consideration of the above several points, the disclosure of WO 93/09806 contains statements that are in error, as well as statements and figures that are inconsistent and uninterpretable, such that one of ordinary skill in the art would not be able to make and use SNO-hemoglobin. Similar statements have been sworn to by Declarants Jonathan S. Stamler, M.D. and Joseph Bonaventura, Ph.D. Therefore, it cannot be said that WO 93/09806 discloses SNO-hemoglobin.

Persons of ordinary skill in the art, taking the specification as a whole and combining this description with their own knowledge of the art, would not be put in possession of SNO-hemoglobin, and from the “results” presented, would not find any reason to try to “optimize” parameters to produce SNO-hemoglobin, as there is no credible indication that any SNO-hemoglobin was produced. Furthermore, there is no source in the scientific literature to suggest a method to “optimize” synthesis parameters of a synthesis method using SNOAc, as no other SNO-protein had been produced by a similar method and no assay method was available to measure a SNO-hemoglobin product. Rather, one of ordinary skill in the art, turning to other methods of the S-nitrosylation of proteins in the literature, would be left with a method using acidified nitrite, which when used on hemoglobin, results in its dissociation into subunits and the loss of oxygen-carrying function.

Rejection of Claims 4, 5 and 9-29 Under 35 U.S.C. § 103(a)

Claims 4, 5 and 9-29 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Stamler *et al.* (WO 93/09806) in view of Feola *et al.*, US 5,439,882, Klatz *et al.*, US 5,385,314 and Hunter, US 5,152,979.

The teachings of Stamler *et al.* (WO 93/09806) have been described above. As discussed above, the teachings of WO 93/09806 are limited, according to the statements in the Declaration of Jonathan S. Stamler and the Declaration of Joseph Bonaventura.

Feola *et al.* (US 5,439,882) describe cross-linked mammalian hemoglobin, a method of making the same, and a method of using the same as a blood substitute. Reduced glutathione (a thiol), is used in this reference in a process of making cross-linked hemoglobin. Glutathione is used to stop the cross-linking of hemoglobin when using o-adenosine as a cross-linking agent; glutathione becomes part of the cross-linked hemoglobin compound. See column 7, line 45-47, wherein the product is described as Hb--P--P--GSH. Also see column 13, lines 2-6 and lines 27-30. The reported function of glutathione is as an "oxidant trap" (column 13, lines 7-14). Note that the composition of Feola *et al.* is dialyzed to prepare it for storage. This step removes all free glutathione. Feola *et al.* do not suggest any role of glutathione in carrying out the biological functions of NO. Feola *et al.* do not teach or suggest any method of therapy by the administration of both hemoglobin and a low molecular weight thiol or nitrosothiol.

Klatz *et al.* (U.S. 5,395,314) describe an apparatus and a method to preserve organs in a cadaver or in a brain-dead patient before the organs can be removed for transplantation. The method employs a solution containing perfluorocarbons, which are to act as a blood substitute and transport oxygen in a manner similar to oxygen transport by hemoglobin. The solution may also contain antioxidants as free radical scavengers.

Klatz *et al.* do not teach or suggest any use of hemoglobin with either a low molecular weight thiol or nitrosothiol. Klatz *et al.* merely suggest the desirability of a blood substitute to supply oxygen to tissues, especially the desirability of a substance to perform this function in excised organs, as in Claims 4 and 22. However, the solution they suggest is an *artificial* oxygen carrier. Hemoglobin, of course, is known to have the desirable characteristic of being an oxygen carrier. Thus, the reference Klatz *et al.* adds nothing to the combination of references. Moreover, with regard to Claims 4 and 22, none of the cited references teaches SNO-hemoglobin or S-nitrosated hemoglobin, and none of the cited references teach or suggest the use of SNO-

hemoglobin in combination with a thiol of any kind, in any method of therapy or method of preservation of an organ. Therefore, no *prima facie* case of obviousness can exist.

Hunter (US 5,152,979) describes a method for treating vascular obstructions, including those which may be caused by infection, sickle cell crisis, malaria and myocardial infarction. The method is to administer to a patient a surface active copolymer of a certain class of hydrophobes to reduce surface tension and friction in blood vessels, thereby reducing the incidence of thrombosis. Hunter may suggest the desirability of some kind of preventive measure against vascular obstructions, but does not teach or suggest the use of any form of hemoglobin. Hunter teaches that the solution to the threat of thrombosis lies not in administering a form of hemoglobin, but in reducing friction between blood cells and the blood vessel surface. Hunter adds nothing to the combination of references.

The combination of references cited is not understood, especially for Claims 9-14, which are drawn to methods for making SNO-hemoglobin and compositions comprising SNO-hemoglobin. As stated above, WO 93/09806 does not teach SNO-hemoglobin. None of the other references cited in this rejection even mention SNO-hemoglobin or imply that it can exist or can be made.

Claims 16, 18, 19, 20, 21, 23, 26, 27, 28 and 29 pertain to the administration of SNO-hemoglobin to a mammal to cause various physiological effects. As explained above, WO 93/09806 does not provide an enabling description of how to make and use SNO-hemoglobin of any kind. None of the other cited references can add anything to make up for this deficiency.

Claims 17, 24 and 25 pertain to the administration of SNO-hemoglobin along with a thiol or S-nitrosothiol, for the purpose of delivering NO or treating a human with sickle cell anemia. None of the cited references, alone or in combination, provides an enabling description of how to make and use SNO-hemoglobin of any kind, for any purpose. Nowhere does WO 93/09806 or any other of the cited references suggest the therapeutic use of a combination of SNO-hemoglobin with anything else.

Claim 5 is drawn to a method of treating malaria, the method including the steps of isolating a patient's red blood cells, treating the red blood cells with S-nitrosothiol, and administering the treated red blood cells to the patient. None of the cited references teaches or suggests any method of treating red blood cells. No *prima facie* case of obviousness can be established with this combination of references.

Claim 15 is drawn to a method for regulating delivery of oxygen and NO, in various redox forms, in a mammal, comprising administering to the mammal an effective amount of a mixture of a low molecular weight thiol or nitrosothiol and hemoglobin or S-nitrosohemoglobin. The cited references do not suggest any of these combinations for any purpose. The Hunter and Klatz references have nothing to do with hemoglobin.

Combining the teachings of the cited references, one of ordinary skill in the art would conclude that trying to produce blood substitutes and therapeutics containing SNO-hemoglobin is a poor idea. One would also conclude that for at least vasodilator effects in a blood substitute, but perhaps not oxygen carrying effects or prevention of thromboses, one should use the cross-linked hemoglobin of Feola *et al.*, because 1) Feola *et al.* teaches that the cross-linking of hemoglobin using o-adenosine produces a hemoglobin product that is a vasodilator; 2) WO 93/09806 does not show the successful synthesis of SNO-hemoglobin; 3) Klatz *et al.* suggest avoiding any form of hemoglobin altogether for use as an oxygen carrier; 4) Hunter suggests no form of hemoglobin, but a kind of "greasing" of the lumen of the blood vessels with polymers to prevent thromboses.

CONCLUSION

The Examiner is respectfully requested to take into consideration the above amendments and remarks, and to withdraw the rejections. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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